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(54) **Title:** 57256 AND 58289, NOVEL HUMAN TRANSPORTERS AND USES THEREOF

(57) **Abstract:** The invention provides isolated nucleic acids molecules, designated MTP or 57256 or 58289 nucleic acid molecules, which encode novel MTP-related transporter molecules. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing MTP nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which an MTP gene has been introduced or disrupted. The invention still further provides isolated MTP or 57256 or 58289 proteins, fusion proteins, antigenic peptides and anti-MTP antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

57256 AND 58289, NOVEL HUMAN TRANSPORTERS AND USES THEREOF

Background of the Invention

Cellular membranes serve to differentiate the contents of a cell from the surrounding environment, and may also serve as effective barriers against the unregulated influx of hazardous or unwanted compounds, and the unregulated efflux of desirable compounds. Membranes are by nature impervious to the unfacilitated diffusion of hydrophilic compounds such as proteins, water molecules, and ions due to their structure: a bilayer of lipid molecules in which the polar head groups face outwards (towards the exterior and interior of the cell) and the nonpolar tails face inwards (at the center of bilayer, forming a hydrophobic core). Membranes enable a cell to maintain a relatively higher intracellular concentration of desired compounds and a relatively lower intracellular concentration of undesired compounds than are contained within the surrounding environment.

However, membranes also present a structural difficulty for cells, in that most desired compounds cannot readily enter the cell, nor can most waste products readily exit the cell through this lipid bilayer. The import and export of such compounds is facilitated by proteins which are embedded (singly or in complexes) in the cellular membrane. There are several general classes of membrane transport proteins: channels/pores, permeases, and transporters. The former are integral membrane proteins which form a regulated passage through a membrane. This regulation, or 'gating' is generally specific to the molecules to be transported by the pore or channel, rendering these transmembrane constructs selectively permeable to a specific class of substrates. For example, a calcium channel is constructed such that only ions having a like charge and size to that of calcium may pass through. Channel and pore proteins tend to have discrete hydrophobic and hydrophilic domains, such that the hydrophobic face of the protein may associate with the interior of the membrane while the hydrophilic face lines the interior of the channel, thus providing a sheltered hydrophilic environment through which the selected hydrophilic molecule may pass. This pore/channel-mediated system of facilitated diffusion is limited to ions and other very small molecules, due to the fact that pore or channels sufficiently large to permit the passage of whole proteins by facilitated diffusion would be unable to prevent the simultaneous passage of smaller hydrophilic molecules.

Transport of larger molecules takes place by the action of 'permeases' and 'transporters', two other classes of membrane-localized proteins which serve to move charged molecules from one side of a cellular membrane to the other. Unlike channel molecules, which permit diffusion-limited solute movement of a particular solute, these proteins require an energetic input, either in the form of a diffusion gradient (permeases) or through coupling to hydrolysis of an energetic molecule (*e.g.*, ATP or GTP) (transporters). The permeases, integral membrane proteins often having between 6-14 membrane-spanning α-helices) enable the facilitated diffusion of molecules such as glucose or other sugars into the cell when the concentration of these molecules on one side of the membrane is greater than that on the other. Permeases do not form open channels through the membrane, but rather bind to the target molecule at the surface of the membrane and then undergo a conformational shift such that the target molecule is released on the opposite side of the membrane.

Transporters, in contrast, permit the movement of target molecules across membranes against the existing concentration gradient (active transport), a situation in which facilitated diffusion cannot occur. There are two general mechanisms used by cells for this type of membrane transport: symport/antiport, and energy-coupled transport, such as

that mediated by the ABC transporters. Symport and antiport systems couple the movement of two different molecules across the membrane (via molecules having two separate binding sites for the two different molecules); in symport, both molecules are transported in the same direction, while in antiport, one molecule is imported while the other is exported. This is possible energetically because one of the two molecules moves in accordance with a concentration gradient, and this energetically favorable event is permitted only upon concomitant movement of a desired compound against the prevailing concentration gradient.

Single molecules may also be transported across the membrane against the concentration gradient in an energy-driven process, such as that utilized by the ABC transporters. In this ABC transporter system, the transport protein located in the membrane has an ATP-binding cassette; upon binding of the target molecule, the ATP is converted to ADP and inorganic phosphate (P_i), and the resulting release of energy is used to drive the movement of the target molecule to the opposite face of the membrane, facilitated by the transporter.

Transport molecules are specific for a particular target solute or class of solutes, and are also present in one or more specific membranes. Transport molecules localized to the plasma membrane permit an exchange of solutes with the surrounding environment, while transport molecules localized to intracellular membranes (*e.g.*, membranes of the mitochondrion, peroxisome, lysosome, endoplasmic reticulum, nucleus, or vacuole) permit import and export of molecules from organelle to organelle or to the cytoplasm. For example, in the case of the mitochondrion, transporters in the inner and outer mitochondrial membranes permit the import of sugar molecules, calcium ions, and water (among other molecules) into the organelle and the export of newly synthesized ATP to the cytosol.

Membrane transport molecules (*e.g.*, channels/pores, permeases, and transporters) play important roles in the ability of the cell to regulate homeostasis, to grow and divide, and to communicate with other cells, *e.g.*, to secrete and receive signaling molecules, such as hormones, reactive oxygen species, ions, neurotransmitters, and cytokines. A wide variety of human diseases and disorders are associated with defects in transporter or other membrane transport molecules, including certain types of liver disorders (*e.g.*, due to defects in transport of long-chain fatty acids (Al Odaib *et al.* (1998) *New Eng. J. Med.* 339: 1752-1757), hyperlysinemia (due to a transport defect of lysine into mitochondria (Oyanagi *et al.* (1986) *Inherit. Metab. Dis.* 9: 313-316)), and cataract (Wintour (1997) *Clin Exp Pharmacol Physiol* 24(1):1-9)).

Summary of the Invention

The present invention is based, at least in part, on the discovery of novel members of the family of transporter molecules, referred to herein as MTP nucleic acid and protein molecules or 57256 or 58289 nucleic acid and protein molecules. 57256 proteins have homology to sugar transporters, as well as glycosyl transferases. 58289 proteins have homology to amino acid transporters, particularly proline transporters. Thus the 57256 or 58289 proteins are expected to function in the transport of sugars or amino acids across the cell membrane. With this functionality, the proteins are expected to be useful upon expression in cells to participate in maintaining homeostasis and supporting cell growth by transporting in necessary substrates.

The present invention also includes the use of 57256 proteins for the transport of various sugars, and the use of 58289 proteins for the transport of amino acids other than proline, under different transport conditions. Furthermore, the present invention also

includes modified versions of the 57256 or 58289 proteins or their encoding nucleic acids to alter their transport specificity for particular sugars and amino acids.

The MTP nucleic acid and protein molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, *e.g.*, cellular proliferation, growth, differentiation, or migration. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding MTP proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of MTP-encoding nucleic acids.

In one embodiment, a 57256 nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:1 or 3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof.

In another embodiment, a 58289 nucleic acid molecule of the invention is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence (*e.g.*, to the entire length of the nucleotide sequence) shown in SEQ ID NO:4 or 6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof.

In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:1 or 3. This cDNA may comprise sequences encoding the human 57256 protein (*i.e.*, "the coding region", from nucleotides 260-1798 of SEQ ID NO:1), as well as 5' untranslated sequences (259 nucleotides before the coding region) and 3' untranslated sequences (323 nucleotides after the coding region) of SEQ ID NO:1. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:1 (*e.g.*, nucleotides 1-1539, corresponding to SEQ ID NO:3).

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:4 or 6. This cDNA may comprise sequences encoding the human 58289 protein (*i.e.*, "the coding region", from nucleotides 159-1802 of SEQ ID NO:4), as well as 5' untranslated sequences (158 nucleotides before the coding region) and 3' untranslated sequences (1965 nucleotides after the coding region) of SEQ ID NO:4. Alternatively, the nucleic acid molecule can comprise only the coding region of SEQ ID NO:4 (*e.g.*, nucleotides 1-1644, corresponding to SEQ ID NO:6.

In another embodiment, a 57256 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In a preferred embodiment, a 57256 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In another embodiment, a 58289 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:5, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In a preferred embodiment, a 58289 nucleic acid molecule includes a nucleotide sequence encoding a protein having an amino acid sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the amino acid sequence of SEQ ID NO:5, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In another preferred embodiment, an isolated nucleic acid molecule encodes the amino acid sequence of human 57256 or 58289. In yet another preferred embodiment, the 57256 nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In yet another preferred embodiment, the 57256 nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides in length. In a further preferred embodiment, the nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides in length and encodes a protein having a 57256 activity (as described herein). In yet another preferred embodiment, the 58289 nucleic acid molecule includes a nucleotide sequence encoding a protein having the amino acid sequence of SEQ ID NO:5, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In yet another preferred embodiment, the 58289 nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350,

400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides in length. In a further preferred embodiment, the nucleic acid molecule is at least 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides in length and encodes a protein having a 58289 activity (as described herein).

Another embodiment of the invention features nucleic acid molecules, preferably 57256 nucleic acid molecules, which specifically detect 57256 nucleic acid molecules relative to nucleic acid molecules encoding non-57256 proteins. For example, in one embodiment, such a nucleic acid molecule is at least 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof. Another embodiment of the invention features nucleic acid molecules, preferably 58289 nucleic acid molecules, which specifically detect 58289 nucleic acid molecules relative to nucleic acid molecules encoding non-58289 proteins. For example, in one embodiment, such a nucleic acid molecule is at least 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:4, the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, or a complement thereof.

In preferred embodiments, the 57256 or 58289 nucleic acid molecules are at least 15 (*e.g.*, 15 contiguous) nucleotides in length and hybridize under stringent conditions to the nucleotide molecules set forth in SEQ ID NO:1 or 4.

In other preferred embodiments, the 57256 nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, or an amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the 57256 nucleic acid molecule hybridizes to a 57256 nucleic acid molecule comprising SEQ ID NO:1 or 3, respectively, under stringent conditions. In other preferred embodiments, the 58289 nucleic acid molecule encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:5, or an amino acid sequence encoded

by the DNA insert of the plasmid deposited with ATCC as Accession Number _____, wherein the 58289 nucleic acid molecule hybridizes to a 58289 nucleic acid molecule comprising SEQ ID NO:4 or 6, respectively, under stringent conditions.

Another embodiment of the invention provides an isolated nucleic acid molecule which is antisense to an MTP nucleic acid molecule, *e.g.*, the coding strand of an MTP nucleic acid molecule.

Another aspect of the invention provides a vector comprising an MTP nucleic acid molecule. In certain embodiments, the vector is a recombinant expression vector. In another embodiment, the invention provides a host cell containing a vector of the invention. In yet another embodiment, the invention provides a host cell containing a nucleic acid molecule of the invention. The invention also provides a method for producing a protein, preferably an MTP protein, by culturing in a suitable medium, a host cell, *e.g.*, a mammalian host cell such as a non-human mammalian cell, of the invention containing a recombinant expression vector, such that the protein is produced.

Another aspect of this invention features isolated or recombinant MTP proteins and polypeptides. In one embodiment, an isolated MTP protein includes at least one or more of the domains necessary for a membrane bound protein (transmembrane domains) and for a sugar or amino acid transport protein.

In a preferred embodiment, a 57256 protein includes at least one or more of the domains necessary for a membrane bound protein (transmembrane domains) and for a sugar transport protein, and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 67%, 68%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:2, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____. In a preferred embodiment, a 58289 protein includes at least one or more of the domains necessary for a membrane bound protein (transmembrane domains) and for an amino acid transport protein and has an amino acid sequence at least about 50%, 55%, 60%, 65%, 67%, 68%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO:5, or the amino acid sequence encoded by the DNA insert of the plasmid deposited with ATCC as Accession Number _____.

In another preferred embodiment, an MTP protein includes at least one or more of the domains necessary for a membrane bound protein (transmembrane domains) and for an amino acid or sugar transport protein and has an MTP activity (as described herein).